

9-16-92

MRID No. 422459-03

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Chlorpyrifos degradate.
Shaughnessey Number: 059101.
- 2. **TEST MATERIAL:** 3,5,6-trichloro-2-pyridinol; CAS No. 6515-38-4; Batch No. AGR 143197; 99.9% active ingredient; a white powder.
- 3. **STUDY TYPE:** Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).
- 4. **CITATION:** Holmes, C.M. and G.J. Smith. 1991. 3,5,6-Trichloro-2-pyridinol: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Laboratory Project No. 103A-104. Performed by Wildlife International Ltd., Easton, Maryland. Submitted by DowElanco, Indianapolis, Indiana. EPA MRID No. 422459-03.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
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Signature: *Rosemary Graham Mora*
Date: 7/14/92

6. **APPROVED BY:**

Louis M. Rifici, M.S.
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Louis M. Rifici
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Signature: P. Kozalwat
for LMR
Date: 7/14/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Date: 9/16/92

- 7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. The EC₅₀ for eastern oysters exposed to 3,5,6-trichloro-2-pyridinol was 9.3 and 10.2 mg a.i./l (mean measured concentrations) when compared to the new shell growth of the dilution water control and solvent control, respectively. Therefore, 3,5,6-trichloro-2-pyridinol is classified as moderately toxic to *Crassostrea virginica*. The NOEC was 4.53 mg a.i./l.

8. **RECOMMENDATIONS:**

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Eastern oysters (*Crassostrea virginica*) were obtained from World's End Aquaculture, Lutherville, Maryland. The oysters appeared to be in good physical condition and showed no signs of stress or disease. The oysters were maintained for 10 days in unfiltered natural seawater with a salinity of 24-25 parts per thousand (ppt), a pH of 7.5, and a temperature of 22.0-22.7°C. Diatoms and green algae were added to provide additional food for the oysters. The oysters had a mean length of 42 mm (range of 37 to 49 mm).

B. Test System: A continuous-flow, proportional diluter was used to provide each selected concentration and control to the test vessels. The test vessels were Teflon®-lined 56-l polyethylene aquaria containing 12.6 l of test solution with a solution depth of 7 cm. A peristaltic pump was used to deliver each stock solution to a mixing chamber where it was mixed with dilution water. One liter of solution/oyster/hour was delivered to each test chamber. The test system was operated for 22.5 hours prior to test initiation.

Test aquaria were randomly positioned in a temperature-controlled water bath maintained at 22 ±1°C. The test was conducted under fluorescent lighting on a 16-hour light (388 lux) and 8-hour dark photoperiod. Thirty-minute dawn and dusk transition periods were provided. Supplemental algae (*Tetraselmis* and *Thalassiosira*) were provided as a supplement to naturally-occurring algae and to maximize shell growth.

The dilution water was natural unfiltered seawater from the Indian River Inlet, Delaware, with a salinity of 23-25 ppt and a pH of 7.4-7.5.

Primary stock solution (0.100 g/ml) was prepared by dissolving the test material with the appropriate amount of methanol. This stock solution was diluted with methanol to prepare four additional stock solutions.

C. Dosage: Ninety-six-hour flow-through acute test. The nominal concentration series selected for this study

was 2.59, 4.32, 7.20, 12.0, and 20.0 mg/l. A dilution water control and a solvent control (0.2 ml/l) were also included.

- D. Design: Immediately prior to test initiation, 3-5 mm of the shell periphery of the oysters were removed. The test was initiated when 20 oysters were impartially selected, by twos, and indiscriminately distributed to each test chamber (one chamber/treatment).

The measurements of new shell growth of each oyster were taken at 96 hours. Dissolved oxygen concentration, salinity, and pH were measured in each chamber at 0, 24, 48, and 96 hours. Temperature was measured in each chamber daily, and was also monitored continuously in the control.

Duplicate samples collected at 0, 48, and 96 hours were analyzed for 3,5,6-trichloro-2-pyridinol using ultraviolet absorbance.

- E. Statistics: The 96-hour EC_{50} and its 95% confidence interval were calculated using inverse prediction. The NOEC was determined using Dunnett's multiple comparison procedure to compare the treatment growth to the pooled control growth data.

12. REPORTED RESULTS: Mean measured concentrations were 2.92, 4.53, 7.74, 12.2, and 20.6 mg a.i./l (Table 1, attached). The measured concentrations averaged 102-113% of nominal concentrations.

Following 96 hours of exposure, the dilution water control and solvent control oysters had a mean new shell growth of 5.07 and 4.62 mm, respectively (Table 3, attached). The 96-hour EC_{50} was 9.3 mg a.i./l with a 95% confidence interval of 3.6-24.2 mg/l. The NOEC was 4.53 mg/l.

During the test period, the pH was 7.7-8.1, the dissolved oxygen concentration ranged from 5.5 to 7.3 mg/l (>60% of saturation), the temperature was 21.7-22.4°C, and the salinity was 25 ppt.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusions, other than those presented above, were made by the author.

A good laboratory practice statement was included in the report, indicating that the study was conducted in

accordance with 40 CFR, Part 160. A Quality Assurance Statement was also included.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure:** The test procedures were generally in accordance with protocols recommended by the SEP, but deviated as follows:

The recommended flow rate is 5 l/oyster/hour; for this study, a rate of 1 l/oyster/hour was provided. However, for this study, it is probably acceptable since supplemental algae were provided and the control oysters met the requirement for new shell growth (2 mm).

The concentration of solvent in the solvent control and exposure concentrations was 0.2 ml/l; a solvent concentration of 0.1 ml/l is recommended for flow-through tests.

The dimensions of the test vessel were not reported.

Pretest mortality was not reported.

- B. Statistical Analysis:** EPA's Toxanal computer program was used to calculate the 96-hour EC_{50} values and its 95% confidence interval using percent growth reduction relative to both the dilution water control and solvent control growth. The 96-hour EC_{50} values (95% confidence interval) compared to the control and solvent control growth were 9.3 (8.6-10) mg a.i./l and 10.2 (9.6-10.7) mg a.i./l, respectively (printouts, attached). These EC_{50} values are the same or similar to the EC_{50} (9.3 mg a.i./l) presented by the authors.

The new shell growth data failed the tests for homogeneity of variance (Hartley test) and normality (chi-square test). The reviewer determined the NOEC using Kruskal-Wallis test. The reviewer's analysis demonstrated a higher NOEC (7.74 mg a.i./l) than that presented by the authors (4.53 mg a.i./l), therefore, the authors' NOEC is accepted.

- C. Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. The EC_{50} for eastern oysters exposed to 3,5,6-trichloro-2-pyridinol was 9.3 and 10.2 mg a.i./l mean measured concentration when comparing the new shell

4

growth to that of the dilution water control and solvent control, respectively. Therefore, 3,5,6-trichloro-2-pyridinol is classified as moderately toxic to *Crassostrea virginica*. The NOEC was 4.53 mg a.i./l.

D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, June 10, 1992.

Table 1.
Summary of Analytical Chemistry Data

Sponsor: The Dow Chemical Company Test Substance: 3,5,6-Trichloro-2-pyridinol Test Organism: Eastern Oyster (<i>Crassostrea virginica</i>) Dilution Water: Natural Unfiltered Saltwater					
Nominal Concentration (mg/L)	Measured Concentration (mg/L)				Percent of Nominal
	0 Hours	48 Hours	96 Hours	Grand Mean	
Negative Control	ND ¹	ND	ND	ND	--
Solvent Control	ND	ND	ND	ND	--
2.59	2.96	3.04	2.77	2.92	113
4.32	4.45	4.71	4.44	4.53	105
7.20	7.82	7.98	7.43	7.74	108
12.0	12.1	12.5	11.9	12.2	102
20.0	20.2	20.8	20.7	20.6	103

¹ ND = Not detected at the Limit of Detection = 1.08 mg/L.

ROSEMARY GRAHAM MORA CHLORPYRIFOS DEGRADATE
(DILUTION WATER CONTROL) EASTERN OYSTER

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
20.6	100	100	100	0
12.2	100	76	76	0
7.74	100	25	25	0
4.53	100	2	2	0
2.92	100	6	6	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.66961

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	1.195014E-02	9.25409	8.611472 9.992483

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.071356	19.10092	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.033121
95 PERCENT CONFIDENCE LIMITS = -.1764779 AND 10.24272

LC50 = 9.105789
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.09318
95 PERCENT CONFIDENCE LIMITS = 0 AND 8.396458

ROSEMARY GRAHAM MORA CHLORPYRIFOS DEGRADATE EASTERN OYSTER (solvent control)

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
20.6	100	100	100	0
12.2	100	73	73	0
7.74	100	17	17	0
4.53	100	0	0	0
2.92	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 10.17428

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	8.831124E-03	10.10483	9.582682 10.66321

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	3.537647E-02	1	.8144924

SLOPE = 8.523061
95 PERCENT CONFIDENCE LIMITS = 6.91999 AND 10.12613

LC50 = 10.16865
95 PERCENT CONFIDENCE LIMITS = 9.643701 AND 10.72639

LC10 = 7.215326
95 PERCENT CONFIDENCE LIMITS = 6.54805 AND 7.756059

8

CHLORPYRIFOS:New Shell Deposition of Exposed Oysters
File: 42245903.oys Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	9.380	33.880	53.480	33.880	9.380
OBSERVED	6	34	62	28	10

Calculated Chi-Square goodness of fit test statistic = 3.6372

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

CHLORPYRIFOS:New Shell Deposition of Exposed Oysters
File: 42245903.oys Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

CHLORPYRIFOS:New Shell Deposition of Exposed Oysters
File: 42245903.oys Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	SOLVENT CONTROL	4.568	4.568	1768.500
2	CONTROL	5.073	5.073	1966.500
3	2.92 mg/l	4.753	4.753	1862.500

9

4	4.53 mg/l	4.965	4.965	1934.000
5	7.74 mg/l	3.817	3.817	1483.500
6	12.2 mg/l	1.243	1.243	615.000
7	20.6 mg/l	0.000	0.000	240.000

Calculated H Value = 89.282 Critical H Value Table = 12.590
 Since Calc H > Crit H REJECT Ho: All groups are equal.

CHLORPYRIFOS: New Shell Deposition of Exposed Oysters
 File: 42245903.oys Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP							
				0	0	0	0	0	0	0	0
				7	6	5	1	3	4	2	
7	20.6 mg/l	0.000	0.000	\							
6	12.2 mg/l	1.243	1.243	.	\						
5	7.74 mg/l	3.817	3.817	*	*	\					
1	SOLVENT CONTROL	4.568	4.568	*	*	.	\				
3	2.92 mg/l	4.753	4.753	*	*	.	.	\			
4	4.53 mg/l	4.965	4.965	*	*	.	.	.	\		
2	CONTROL	5.073	5.073	*	*	\	

* = significant difference (p=0.05)
 Table q value (0.05,7) = 3.038

. = no significant difference
 SE = 12.796

TITLE: CHLORPYRIFOS: New Shell Deposition of Exposed Oysters
 FILE: 42245903.oys
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	5.7500	5.7500
1	SOLVENT CONTROL	2	6.0500	6.0500

10

1	SOLVENT CONTROL	3	6.7000	6.7000
1	SOLVENT CONTROL	4	3.2000	3.2000
1	SOLVENT CONTROL	5	4.4500	4.4500
1	SOLVENT CONTROL	6	5.3500	5.3500
1	SOLVENT CONTROL	7	2.0000	2.0000
1	SOLVENT CONTROL	8	3.6500	3.6500
1	SOLVENT CONTROL	9	4.8500	4.8500
1	SOLVENT CONTROL	10	7.5500	7.5500
1	SOLVENT CONTROL	11	4.5500	4.5500
1	SOLVENT CONTROL	12	2.0000	2.0000
1	SOLVENT CONTROL	13	5.2500	5.2500
1	SOLVENT CONTROL	14	7.9000	7.9000
1	SOLVENT CONTROL	15	4.6500	4.6500
1	SOLVENT CONTROL	16	2.9000	2.9000
1	SOLVENT CONTROL	17	4.2500	4.2500
1	SOLVENT CONTROL	18	3.1500	3.1500
1	SOLVENT CONTROL	19	3.5500	3.5500
1	SOLVENT CONTROL	20	3.6000	3.6000
2	CONTROL	1	5.1000	5.1000
2	CONTROL	2	2.2000	2.2000
2	CONTROL	3	6.4500	6.4500
2	CONTROL	4	3.4500	3.4500
2	CONTROL	5	6.9500	6.9500
2	CONTROL	6	4.5500	4.5500
2	CONTROL	7	8.7500	8.7500
2	CONTROL	8	4.6500	4.6500
2	CONTROL	9	6.8000	6.8000
2	CONTROL	10	6.2000	6.2000
2	CONTROL	11	4.5500	4.5500
2	CONTROL	12	5.0000	5.0000
2	CONTROL	13	4.4000	4.4000
2	CONTROL	14	7.8000	7.8000
2	CONTROL	15	4.7500	4.7500
2	CONTROL	16	2.8000	2.8000
2	CONTROL	17	3.9500	3.9500
2	CONTROL	18	4.2000	4.2000
2	CONTROL	19	4.2500	4.2500
2	CONTROL	20	4.6500	4.6500
3	2.92 mg/l	1	4.0500	4.0500
3	2.92 mg/l	2	3.3500	3.3500
3	2.92 mg/l	3	5.1500	5.1500
3	2.92 mg/l	4	2.2000	2.2000
3	2.92 mg/l	5	3.5500	3.5500
3	2.92 mg/l	6	5.9500	5.9500
3	2.92 mg/l	7	2.6000	2.6000
3	2.92 mg/l	8	4.5000	4.5000
3	2.92 mg/l	9	5.7500	5.7500
3	2.92 mg/l	10	5.8000	5.8000
3	2.92 mg/l	11	4.6000	4.6000
3	2.92 mg/l	12	3.2500	3.2500
3	2.92 mg/l	13	3.2500	3.2500
3	2.92 mg/l	14	4.2000	4.2000
3	2.92 mg/l	15	5.6500	5.6500
3	2.92 mg/l	16	5.1000	5.1000
3	2.92 mg/l	17	7.5500	7.5500
3	2.92 mg/l	18	7.3500	7.3500
3	2.92 mg/l	19	6.0500	6.0500
3	2.92 mg/l	20	5.1500	5.1500
4	4.53 mg/l	1	2.9500	2.9500
4	4.53 mg/l	2	4.0500	4.0500

4	4.53 mg/l	3	3.7000	3.7000
4	4.53 mg/l	4	5.8500	5.8500
4	4.53 mg/l	5	7.6000	7.6000
4	4.53 mg/l	6	3.5500	3.5500
4	4.53 mg/l	7	7.9500	7.9500
4	4.53 mg/l	8	4.7500	4.7500
4	4.53 mg/l	9	5.9000	5.9000
4	4.53 mg/l	10	5.1500	5.1500
4	4.53 mg/l	11	4.3000	4.3000
4	4.53 mg/l	12	5.6500	5.6500
4	4.53 mg/l	13	5.7500	5.7500
4	4.53 mg/l	14	6.1500	6.1500
4	4.53 mg/l	15	2.2000	2.2000
4	4.53 mg/l	16	6.7000	6.7000
4	4.53 mg/l	17	4.0000	4.0000
4	4.53 mg/l	18	5.7500	5.7500
4	4.53 mg/l	19	3.5500	3.5500
4	4.53 mg/l	20	3.8000	3.8000
5	7.74 mg/l	1	2.4000	2.4000
5	7.74 mg/l	2	1.1500	1.1500
5	7.74 mg/l	3	4.0500	4.0500
5	7.74 mg/l	4	3.4500	3.4500
5	7.74 mg/l	5	3.9000	3.9000
5	7.74 mg/l	6	5.0000	5.0000
5	7.74 mg/l	7	3.4500	3.4500
5	7.74 mg/l	8	3.8000	3.8000
5	7.74 mg/l	9	4.1500	4.1500
5	7.74 mg/l	10	2.4500	2.4500
5	7.74 mg/l	11	3.7500	3.7500
5	7.74 mg/l	12	5.0000	5.0000
5	7.74 mg/l	13	5.2000	5.2000
5	7.74 mg/l	14	4.2000	4.2000
5	7.74 mg/l	15	4.1500	4.1500
5	7.74 mg/l	16	3.2500	3.2500
5	7.74 mg/l	17	3.3000	3.3000
5	7.74 mg/l	18	4.4500	4.4500
5	7.74 mg/l	19	5.1000	5.1000
5	7.74 mg/l	20	4.1500	4.1500
6	12.2 mg/l	1	2.5500	2.5500
6	12.2 mg/l	2	0.0000	0.0000
6	12.2 mg/l	3	1.6000	1.6000
6	12.2 mg/l	4	1.2000	1.2000
6	12.2 mg/l	5	2.0000	2.0000
6	12.2 mg/l	6	1.0000	1.0000
6	12.2 mg/l	7	1.8500	1.8500
6	12.2 mg/l	8	2.4000	2.4000
6	12.2 mg/l	9	2.8000	2.8000
6	12.2 mg/l	10	1.7000	1.7000
6	12.2 mg/l	11	1.3000	1.3000
6	12.2 mg/l	12	0.8500	0.8500
6	12.2 mg/l	13	0.4000	0.4000
6	12.2 mg/l	14	0.5000	0.5000
6	12.2 mg/l	15	0.0000	0.0000
6	12.2 mg/l	16	0.5500	0.5500
6	12.2 mg/l	17	2.1000	2.1000
6	12.2 mg/l	18	1.3500	1.3500
6	12.2 mg/l	19	0.0000	0.0000
6	12.2 mg/l	20	0.7000	0.7000
7	20.6 mg/l	1	0.0000	0.0000
7	20.6 mg/l	2	0.0000	0.0000

12

7	20.6 mg/l	3	0.0000	0.0000
7	20.6 mg/l	4	0.0000	0.0000
7	20.6 mg/l	5	0.0000	0.0000
7	20.6 mg/l	6	0.0000	0.0000
7	20.6 mg/l	7	0.0000	0.0000
7	20.6 mg/l	8	0.0000	0.0000
7	20.6 mg/l	9	0.0000	0.0000
7	20.6 mg/l	10	0.0000	0.0000
7	20.6 mg/l	11	0.0000	0.0000
7	20.6 mg/l	12	0.0000	0.0000
7	20.6 mg/l	13	0.0000	0.0000
7	20.6 mg/l	14	0.0000	0.0000
7	20.6 mg/l	15	0.0000	0.0000
7	20.6 mg/l	16	0.0000	0.0000
7	20.6 mg/l	17	0.0000	0.0000
7	20.6 mg/l	18	0.0000	0.0000
7	20.6 mg/l	19	0.0000	0.0000
7	20.6 mg/l	20	0.0000	0.0000
